35 U.S.C. §112, second paragraph

In Paper No. 5, the Examiner rejected the claims for indefiniteness, stating:

> the use of Jepson format is confusing, as methods of cloning are not broadly enabled by the art. It would be clearer if the claimes were to be written in regular format clearly stating at which step of the known methods is the claimed method to be inserted.

Accordingly, the new claims are written in conventional format to more clearly define the subject matter being claimed. This rejection can now be withdrawn.

35 U.S.C. §112

Claims 87-141 were rejected for lack of enablement. On pages 5 of Paper No. 16, the Examiner states:

> The skilled artisan would need to engage in an undue amount of experimentation without a predictable degree of success to implement the invention as claimed as there is insufficient guidance in the specification, in view of the state of the art at the time of filing, that the claimed methods would result in nuclear reprogramming sufficient to produce by nuclear transfer a viable term animal.

This rejection is traversed.

Applicant has made a significant contribution to the field of whole animal cloning by discovering a critical element of the reprogramming process.

The art of nuclear transplantation for the purpose of cloning was already well-developed at the time of the invention (see, e.g., page 19, lines 5-11, of the specification, describing cloning of tadpoles). Difficulties in cloning animals had to do with reprogramming somatic cell nuclei. The solution to these difficulties was discovered by the Applicant – contacting somatic cell nuclei with a meiotic metaphase II cytoplasm prior to activation of the nuclei was key to reprogramming (i.e., eliminating somatic cell patterns of gene structure and function such as methylation patterns). The fundamental and unique property of a metaphase II cytoplasm was the discovery that provided the solution to earlier problems associated with cloning of whole animals.

Applicant submits that the claimed methods would not require undue experimentation because, other than the reprogramming step, the state of the art of nuclear transplantation was

well established at the time of the invention. In discovering the role of a cystostatic factor – containing composition, i.e., metaphase II cytoplasm, a major inadequacy of first generation cloning technology was overcome. Contacting a somatic cell nucleus with a metaphase II cytoplasm is a simple step, which is described in detail in the present specification. Other steps of the procedure were known in the art of nuclear transplantation cloning. Given this backdrop, one of skill in the art would not have to resort to undue experimentation to carry out the claimed methods.

Enablement Standard

Enablement is not precluded by the necessity for some experimentation; however, any required experimentation must not be undue experimentation. Applicant submits that the specification coupled with the knowledge in the art of nuclear transplantation cloning fulfills the requirements of §112 for enablement. Undue experimentation would not be required to practice the claimed methods.

The factors to be analyzed in determining whether undue experimentation is required to practice the full scope of the claims are discussed in In re Wands.[1] The court in In re Wands set forth eight factors to be considered in determining whether undue experimentation would be required: (1) the state of the prior art, (2) the predictability or unpredictability of the art, (3) the breadth of the claims (4) the presence or absence of working examples, (5) the amount of direction or guidance presented, (6) the relative skill of those in the prior art, (7) the nature of the invention, and (8) the quantity of experimentation necessary.

Nature of the invention and state of the prior art

The nature of the invention is nuclear activation/transplantation.

With regard to the state of the art, Applicant submits that the art of nuclear transplantation and cloning was active and established at the time of the invention (page 18, line 30, to page 19,

[1] In re Wands, 858 F.2d 731, 736-7 (Fed. Cir. 1988).

line 11; and page 59, line 26, to page 60, line 6, of the specification). Earlier stumbling blocks were in the "reprogramming step" – prior to the invention, a critical element of the cloning process was missing – i.e., how to treat a somatic cell nucleus to initiate development of a new organism under the direction of genetic information contained in a transplanted nucleus. Applicant provided this missing element, and thereby, revolutionized the art.

Predictability or unpredictability of the art

As was discussed above, methods for nuclear transplantation were standard and predictable at the time of the invention. The methods, e.g., methods described by DiBerardino et al. (page 19, lines 5-11, of the specification), predictably yielded live animals and supported advanced organism development. With regard to reactivation or reprogramming, U.S.P.N 5,480,772 reports that nuclei pretreated with CSF (metaphase II cytoplasm) activate to a greater extent upon exposure to an activating condition than those nuclei that were not pretreated (col. 5, lines 5-10). Moreover, the data in Applicant's publication entitled "Efficient reactivation of *Xenopus* erythrocyte nuclei in *Xenopus* egg extracts" (Wangh et al., 1995, J. Cell Sci. 108:2187-2196; of record as Exhibit 6 of Declaration of Professor Wangh submitted on November 21, 2000) further indicates that contacting nuclei with a metaphase II cytoplasm prior to activation with an activating cytoplasm predictably and efficiently reprograms the nuclei to an activated state. With Applicant's contribution of the "winning formula", i.e., metaphase II cytoplasm, there is no reason to believe that the methods as described in the specification would not predictably reprogram a nucleus to direct development of a cloned organism after transplantation into a recipient egg, as claimed.

Breadth of the claims

The claims have been amended to require contacting a somatic cell nucleus with a cytostatic factor-containing cytoplasm of a cell in meiotic metaphase II prior to activating the nucleus. The requirement of exposing a nucleus to a metaphase II cytoplasm (non-activating condition) before exposing the nucleus to an egg cytoplasm prior to S-phase (activation

condition) represents a breakthrough in the technology of nuclear transplantation and cloning. This fundamental scientific discovery is described in the specification, and the amended claim language now specifically requires this critical step of the cloning process. Applicant submits that the scope of the amended claims is commensurate with the scope of Applicant's contribution to the field of nuclear transplantation cloning and the scope of the disclosure provided in the specification.

Presence or absence of working examples

The specification describes several examples in which non-dividing nuclei were contacted with a cytostatic factor-containing cytoplasm of a cell in meiotic metaphase II prior to activating the nucleus. The examples describe successful reprogramming as demonstrated by DNA replication and entry into mitosis using (a) *Xenopus* erythrocyte nuclei (Example 10, page 72, line 25, to page 74, line 7, of the specification); (b) nuclei from human fetal red blood cells (Example 11, page 74, line 8, to page 75, line 36, of the specification), and (c) human fetal liver cells (Example 12, page 76, line 1, to page 81, line 21, of the specification.

The reprogramming or activation of a somatic (non-dividing) cell nucleus is the key to successful cloning of a whole organism. The data described in the Declaration of Alexander Baguisi indicates that contacting non-dividing nuclei with a metaphase II cytoplasm and then proceeding with standard cloning methods (introducing reconstructed embryos into pseudo-pregnant mice) leads to live cloned animals produced under direction of genetic material of the transplanted nucleus.

In view of the examples of nuclear activation provided in the specification and the example of successful production of cloned animals using the activated nuclei, Applicant submits that the specification and subsequent data indicate that undue experimentation is not required to practice the claimed invention.

Amount of direction or guidance presented

The specification describes successful activation of non-dividing somatic cell nuclei using several different cell types and provides a description of how to clone whole animals using activated nuclei. Methods for obtaining and preparing non-dividing nuclei for nuclear activation is described at page 20, line 33, to page 25, line 5, of the specification. An activating egg cytoplasm is described as being at a point in the cell cycle prior to S-phase (page 25, lines 22-24, and page 30, lines 24-29, of the specification). Cytostatic factor-containing cytoplasm of a cell in meiotic metaphase II is described at page 34, line 14, to page 39, line 26, of the specification.

On page 4, lines 13-15, of Paper No. 16, the Examiner states, "The examiner could find no reference where the nuclei were incubated in CSF egg extract and then activating egg extract". The specification teaches that nuclear activation is increased by pretreating with CSF (metaphase II) egg cytoplasm followed by activating (prior to S-phase) egg cytoplasm (page 38, lines 27-30; page 78, lines 1-34, of the specification).

Reprogramming or activation of the non-dividing nuclei is evaluated using a number of different indices such as nuclear swelling, DNA synthesis, or entry into mitosis. For example, DNA replication or synthesis is measured by detecting incorporation of labeled nucleotides (page 41, lines 22-34; page 47, lines 11-19, of the specification.

Methods of cloning whole animals by nuclear transplantation is described on page 58, line 19, to page 60, line 26, of the specification. Details regarding isolation of nuclei, pretreatment, activating treatment, and nuclear transfer are described in sufficient detail to instruct one skilled in the relevant art to activate nuclei, transplant them into recipient cells, and grow the cells to produce a cloned whole animal.

Applicant therefore submits that the level of guidance presented in the specification is sufficient for one skilled in the art to carry out the claimed methods without undue experimentation.

Relative skill of those in the prior art

The skilled artisan in the relevant field is a cell biologist. The average cell biologist has an advanced degree with several years of experience in manipulating cells and subcellular organelles and components. As was discussed above, the hurdles in nuclear transplantation and cloning at the time of the invention had to do with how to effectively reprogram a non-dividing cell to an activated state, e.g., a state characterized by DNA synthesis. Given the high level of skill in the art, the level of disclosure provided in the specification is sufficient for the average artisan to practice the claimed invention without undue experimentation.

Quantity of Experimentation Necessary

In Wands, the Court stated,

> [A] considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed.

Applying this criterion here, all of the techniques required to practice the claimed methods were described in the specification or were known to those skilled in the art as of the filing date.

Once the critical composition (metaphase II cytoplasm) was identified, all the tools were available to practice the invention. The specification describes successful nuclear activation using several different cell types. Given the nuclear activation data described in the specification and the data showing that nuclei activated according to the invention are successfully used to clone whole animals, Applicant submits that the undue experimentation would not be required of one skilled in the art to practice the claimed invention.

CONCLUSION

On the basis of the foregoing amendments, Applicant respectfully submits that the pending claims are in condition for allowance. If there are any questions regarding these amendments and remarks, the Examiner is encouraged to contact either of the undersigned at the telephone number provided below.

A petition for extension of time and a check in the amount of $465.00 is enclosed to cover the petition fee for a three month extension of time pursuant to 37 C.F.R. § 1.17(a)(3). A Request for Continuing Examination and a check in the amount of $375.00 is also enclosed The Commissioner is hereby authorized to charge any additional fees that may be due, or credit any overpayment of same, to Deposit Account No. 50-0311, Reference No. 21578-010.

Respectfully submitted,

IA Beattie

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Dated: February 24, 2003

EXHIBIT A

Marked up Version

Cancel claims 87-141. Add new claims 157-163.

--157. A method for reprogramming a non-dividing nucleus, comprising contacting said nucleus with a cytostatic factor-containing cytoplasm of a cell in meiotic metaphase II, wherein said nucleus is reprogrammed to undergo nuclear swelling, nucleic acid replication, and entry into mitosis.--

--158. The method of claim 157, further comprising contacting said nucleus with an activating egg cytoplasm.--

--159. The method of claim 158, wherein said activating egg cytoplasm is at a time in the cell cycle prior to cell cycle S-phase.--

--160. The method of claim 157, wherein said nucleus is reprogrammed to undergo DNA replication.--

--161. The method of claim 157, wherein said egg cytoplasm comprises an activation activity of 70% or greater of peak activation.--

--162. A method for reprogramming a somatic cell nucleus for transplantation into an egg, comprising

contacting said nucleus with an cytostatic factor-containing cytoplasm of a cell in meiotic metaphase II prior to activating said nucleus;

transplanting said nucleus into an enucleated recipient egg;

wherein said nucleus is reprogrammed to direct development of a cloned organism after transplantation into said recipient egg.--

--163. A method for *in vitro* activation of a non-dividing nucleus, comprising the steps of:

(b) providing an isolated somatic cell nucleus;

(b) pretreating said isolated nucleus with a cytostatic-factor containing cytoplasm to yield a pretreated nucleus; and

(d) contacting said pretreated nucleus with an activating egg cytoplasm;

wherein said pretreated nucleus is activated to undergo DNA replication.--